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DECLARATION UNDER 37 C.F.R. §1.131 Address to: Commissioner for Patents P.O. Box 1450 Alexandria VA 22313-1450	Attorney Docket	CLON-056CIP
	Confirmation No.	2176
	First Named Inventor	Tchaga, Grigoriy S.
	Application Number	09/858,332
	Filing Date	May 15, 2001
	Group Art Unit	1652
	Examiner Name	Kathleen M. Kerr
	Title	*POLYNUCLEOTIDES ENCODING METAL ION AFFINITY PEPTIDES AND RELATED PRODUCTS*

Sir:

This Declaration and the attached Exhibit are being submitted in conjunction with the Applicants' Response to the Office Action dated May 6, 2005.

We, Grigoriy S. Tchaga, and George G. Jokhadze, do hereby declare as follows:

1. We are the listed co-inventors of the above-captioned application.

2. Enclosed with this declaration is Exhibit A that provides evidence of conception and reduction to practice of the claimed invention prior to July 1998.

3. Specifically, Exhibit A is a series of laboratory notebook pages, all of which were in existence prior to July 1998), that describe incorporation of a polyhistidine metal ion affinity sequences at the terminal sequence of a recombinant DHFR protein and use of the affinity sequence for the purification of the recombinant DHFR protein with high selectivity.

a. Page 1 of Exhibit A briefly describes the purification protocol for the HAT-DHFR using FPLC under native conditions and also by batch-gravity flow under denaturing conditions.

b. Page 2 of Exhibit A shows preliminary purification results for the HAT-DHFR.

c. Page 3 of Exhibit A is a letter from ATG Laboratories, the contract laboratory employed to clone the HAT-DHFR fusion polypeptide. The letter described in detail the procedure used for cloning the DHFR gene into the KpnI site of the pHS20 vector in order to generate the HAT-DHFR fusion polypeptide.

d. Page 4 of Exhibit A provides the N-terminal portion of the nucleic acid and amino acid sequence of the HAT-DHFR fusion protein encoded vector generated by ATG Laboratories. The amino acid sequence of the metal ion affinity tag is shown in the last two rows and is highlighted and shown in reverse order.

4. The disclosure appearing in Exhibit A clearly shows conception and reduction to practice of the claimed invention of the present application.

5. Accordingly, the evidence provided in Exhibit A establishes that the claimed invention of the above captioned application was conceived and reduced to practice prior to July 1998.

6. We do hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by

fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully submitted,

Date: 7/7/05

Grigoriy S. Tchaga

Date: 7/1/05

George G. Jokhadze

Attachments: Exhibit A

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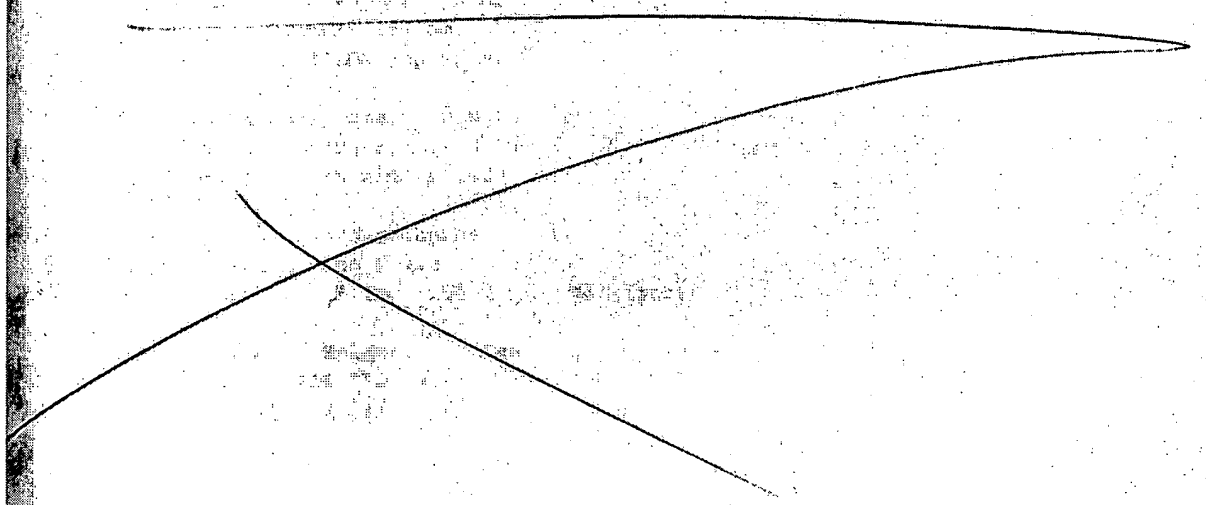
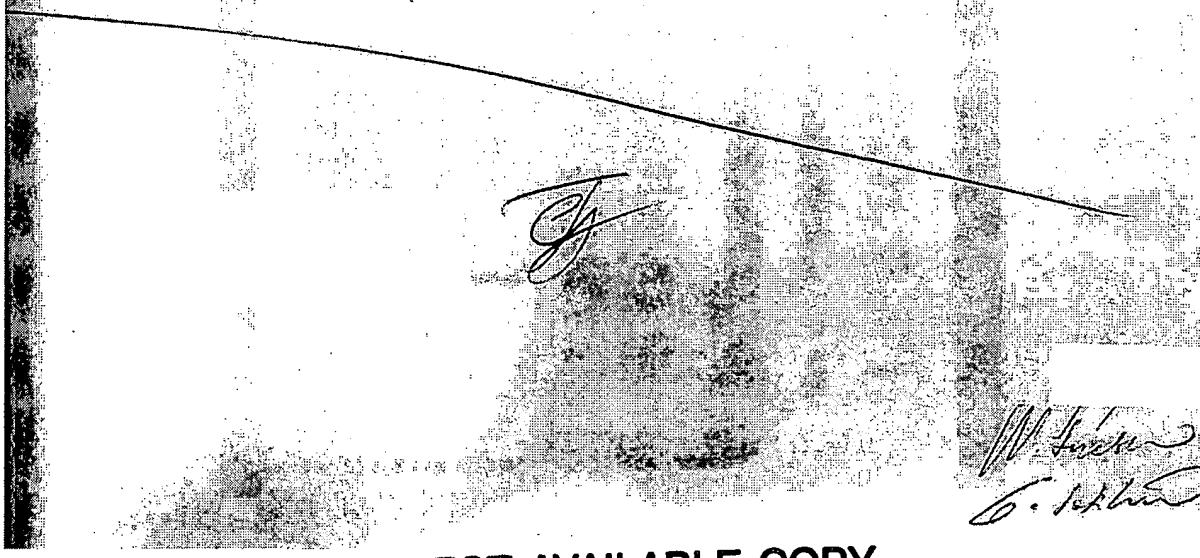
Exhibit A

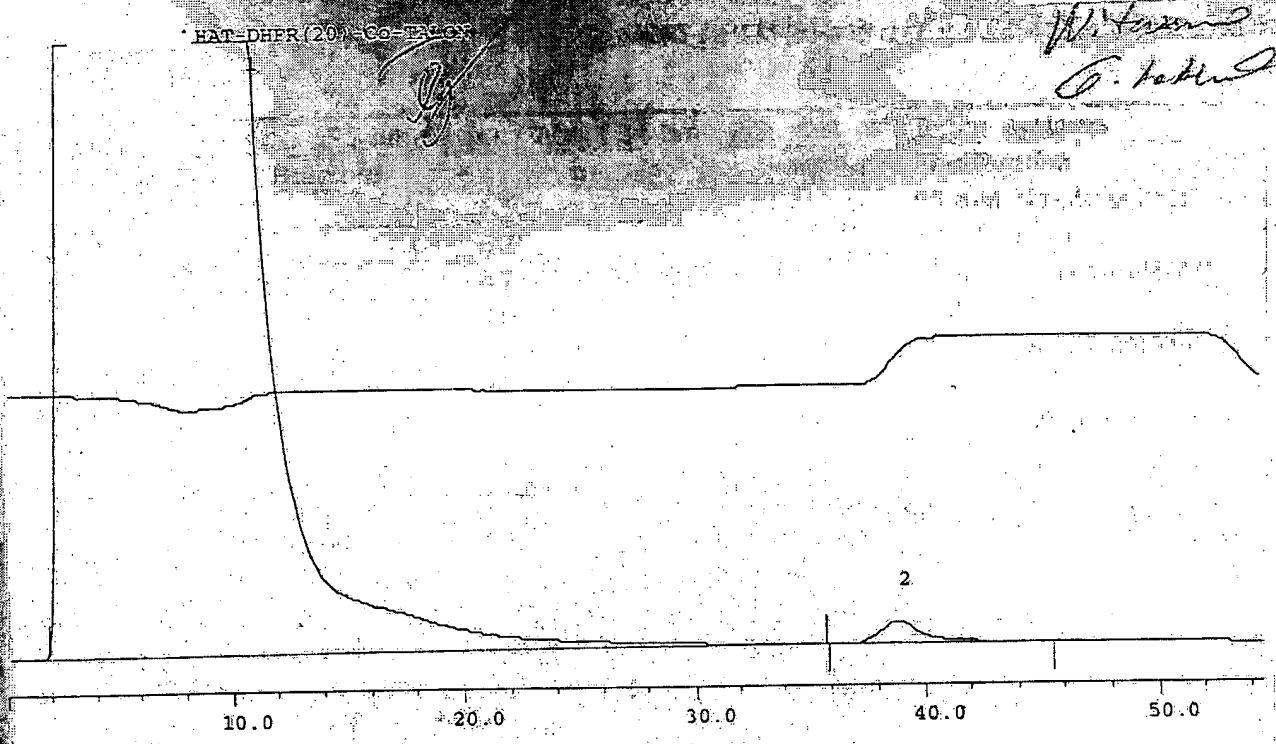
1. Writing HAT manual
2. Purification of HAT-DHFR (20 mer) by FPLC under native and by batch/gravity flow under denaturing conditions.

A. FPLC
Load at 0 mM imidazole, wash at 5 mM imidazole and elution at 150 mM imidazole.
Sample: 300 mg of cells
Flow rate: 1 mL/min.
Fraction size: 1 mL
Quite good recovery and purity, similar to that of the 32 mer HAT-GFPuv.

B. Batch/gravity
Purification protocol - the same as for the FPLC.
Sample: 100 mg of cells.
Obs! Binding at 6 M Guanidinium.HCl not quite strong as the one with the 32 mer - the HAT-DHFR is eluted with 5 mM imidazole (more than 70 %) but even there it is very pure.
The same purification should be repeated with wash at 0 mM imidazole and immediate elution at 150 mM imidazole.



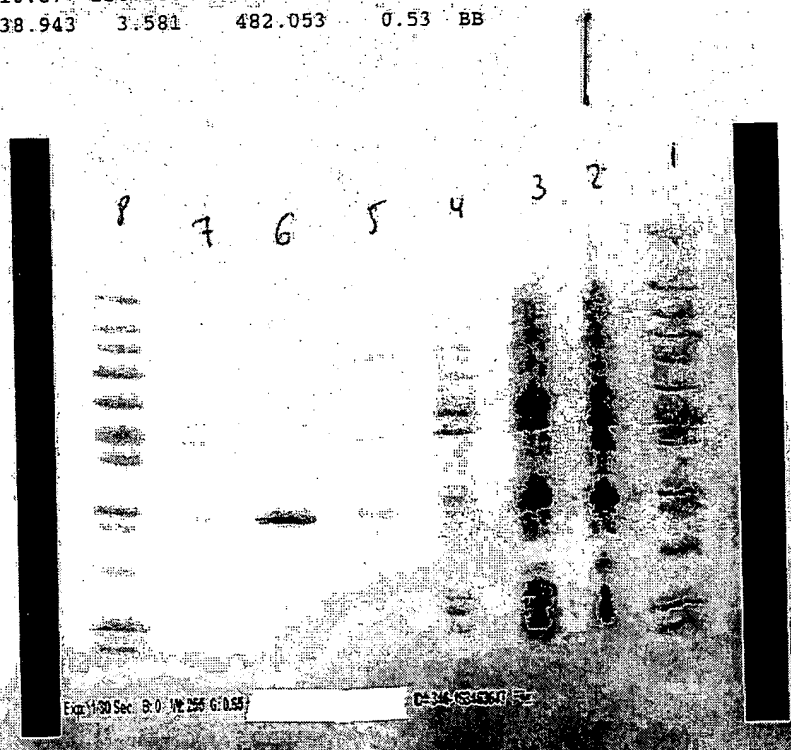




Sample: HAT-DHFR 20-mer - 2 mL
Mon. sens. 2.0 AUFS 254 nm
Column: Co(II)-TALON Superflow 6 3.5x1.0 cm.i.d.
Rate: 1 mL/min
A. 50 mM Na-phosphate; 0.3 M NaCl pH 7.0
B. 20 mM Na-phosphate; 0.3 M NaCl; 150 mM imidazole pH 7.0
Run: B=3.3% for 35 min; B=100% for 15 min

5.000000 Samples/Sec/Channel
Manual Baseline and Peaks
Abs. 254 nm -156.2 to 156.2 Millivolts

Peak	Time	Height	Area	%	Type
1	10.877	156.286	91314.749	99.47	BB++
2	38.943	3.581	482.053	0.53	BB



1. MW st.
2. SDS extr. cell Before Ind.
3. OS
4. Non ads.
5. Wash x10
6. HAT-DHFR x5
7. OS x10
8. MW st.

ATG™
LABORATORIES, INC.
Custom Cloning and Expression Services

TO: Paul Nelson, Ph.D.
Clontech Laboratories, Inc.

FROM: Laura T. Kakach

Final Report: Cloning DHFR gene into pHS20

The purpose of this project was to generate by PCR the DHFR gene from pQE40w and clone it into the KpnI site of pHS20. The strategy used is described.

The PCR primers were designed to amplify the coding region of the DHFR gene in pQE40w (without the ATG at the front end) to the Kpn I site present in the plasmid. All primers were purchased from Life Technologies, Inc. (Gaithersburg, MD). The front end primer, P57-S138 contains a KpnI site just upstream of the start of the DHFR gene to facilitate cloning. The Project Primer Catalog Report which is included with this report shows all the primers used for this project. In addition, the map of pQE40w is included and the primer locations are indicated on the map.

The PCR was carried out in duplicate using pQE40w as a template and the PCR primers P57-S138 and P57-A737. PCR products of the expected size (599 bp) were generated in both reactions. The PCR reactions were extracted with phenol, then chloroform, and ethanol precipitated. The DNA was then subjected to restriction digestion with KpnI and a fragment of 578 bp was gel purified. This fragment was cloned into the KpnI site of pHS20. Transformants were screened for the presence of a 578 bp KpnI fragment, as well as a 326 bp SacI fragment. One clone from each independent PCR reaction was chosen for sequence analysis. The clones were named pHSDHFR-A3 and pHSDHFR-B1. A map of pHS20 (named pUCHS20.MPD) is included.

Four sequencing reactions were carried out for each clone. Full sequence analysis was carried out for the A3 clone. The identification of the sequencing reactions is included with the report. The sequence of the DHFR gene in the A3 clone matched the sequence of the DHFR gene in the pQE40w plasmid with one exception. Nucleotide 606 on the map named PHSDHFR2 is an A, but both clones showed the base to be a G. This nucleotide change is silent in the DHFR gene, the affected codon is CTT to CTC, which both encode leucine. The sequence project data is included, named DHFRA3.SQD. The map of the plasmid is also included, named PHSDHFR2.MPD.

Finally, a photograph is included showing both A3 and B1 clones digested each with KpnI and SacI. The KpnI digests show a fragment approximately 575 bp as expected. The SacI digests show both clones to be in the orientation drawn in the map, which is in the same orientation as the lacZ coding region.

HSDHFR2.MPD (1 > 3335) Site and Sequence